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New ideas for teaching science

AP® Biology Lab 6a

Bacterial Transformation

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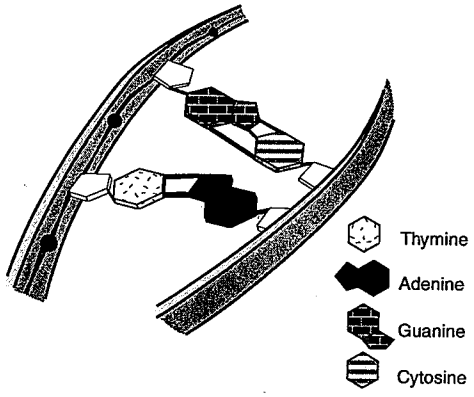
Background

What Is Biotechnology?

Before you start doing biotechnology laboratory exercises, it is important to know exactly what biotechnology is and why it is useful. The first part of the word — "bio" — means "living." The second part of the word — "technology" — means "the practical application of knowledge." Thus biotechnology can be defined as the practical application of knowledge acquired from the study of living things.

Many of these applications are not new to us. People have been making food, medicines, and other products using the unique properties of living organisms for centuries. Ancient Egyptians knew how to make wine and beer by fermenting grapes and grains. For centuries, humans have taken advantage of the characteristics of living organisms to make cheese and bread. We have known that it is possible to create corn that grows larger ears and cows that produce more milk by selectively breeding species with particular combinations of traits.

What's new about biotechnology is our understanding. Ancient Egyptians and early farmers didn't really know why and how fermentation or selective breeding worked. They learned by trial and error. Today, scientists understand what happens at each step of the complex biological and chemical process. These processes are carried out according to instructions provided by each cell's deoxyribonucleic acid (DNA).



Double Helix DNA

Once scientists understand the genetic code that regulates cell activity, they can look for ways to alter and manipulate cellular functions to manufacture specific products in a predictable and controllable way.

Bacterial Transformation

The scientific revolution in biology which started in 1973 by biochemists Stanley Cohen and Herbert Doyer, yielded techniques that enabled biologists to produce genetically engineered organisms. The two biochemists isolated a gene from the toad, Xenopus laevis, and transferred it to the bacterium Escherichia coli. The process of isolating genes from one organism, building recombinant DNA molecule and inserting it into another organism is a field known as genetic engineering. The benefits of genetic engineering has been evident in agriculture, medicine, environment and many other areas. For example, most people diagnosed as having diabetes need to take a hormone called insulin. The old way of making this drug was to extract it from animal pancreatic glands followed by purification. Today the human gene for making insulin can be spliced into a plasmid which is used as a vehicle to transport it into bacteria cells which act as the host. The bacterial cells then expresses the insulin gene as its own and produce insulin.

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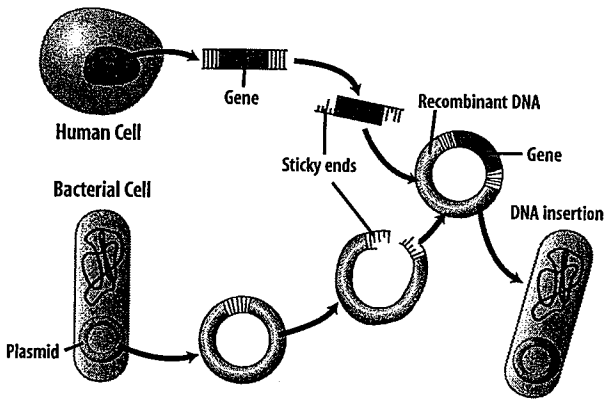
To insert a gene into a plasmid, restriction enzymes derived from bacteria are first used to cut apart the gene of interest. These enzymes recognize specific nucleotide sequences and cut the DNA at these sites into fragments with short "sticky ends." Complementary sticky ends from different organisms can then be joined together to form a recombinant DNA molecule.



Bacterial Transformation

Bacterial cells contain a single chromosome, but in order to ensure survival, more information is usually needed than what is provided by the chromosome. This additional information is encoded in plasmid DNA. Plasmids are "mini-chromosome," small circular DNA molecules found mostly in bacteria but also in select yeast strains. They contain genes that are useful to the host, as well as genes that encode for antibiotic resistance. Such genes produce large amounts of enzymes that chemically neutralize the antibiotics. At any time, a spontaneous mutation can occur at a plasmid in the bacterial cell, which could give rise to an antibiotic resistant gene.

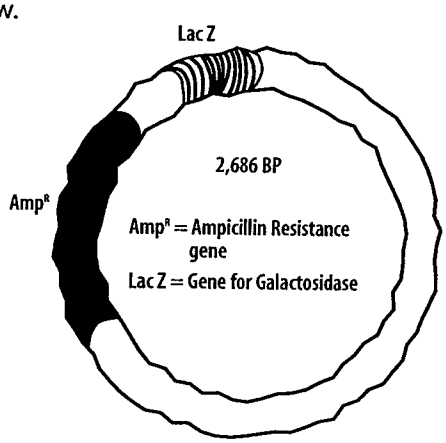
During conjugation or bacterial mating, the plasmids replicate and are transferred between bacteria. This conjugation, or transfer of plasmids between bacteria, make bacteria antibiotic resistant and thus more difficult for doctors to treat an infection.

When microbes began resisting penicillin, scientists developed new antibiotics to fight them off. However, today scientists fear that they have reached near the end of new antibiotic development. The latest developed antibiotic is Vancomycin, known as the drug of "last resort". Some hospital staph (Staphylococcus aureus) infections are resistant to all known antibiotics, except vancomycin. However, vancomycin resistance has turned up in another commonly hospital found bacteria, enterococcus. And since we know that bacteria exchange resistant genes quite rapidly, it is only a matter of time that Vancomycin-resistant staph infections appear. Staphylococcus may pick up vancomycin resistant genes from enterococci, which are found in the normal gut.

Lab Overview

This laboratory provides an introduction to some of the techniques used in the field of genetic engineering. The laboratory investigation demonstrates the process of bacterial transformation using a plasmid that confers ampicillin antibiotic resistance to *E. coli.*

The plasmid that will be used to transform bacteria in this investigation is pUC18 and occurs naturally in *E.coli.* It contains an ampicillin resistant gene that confers antibiotic resistance to bacterial cells. Transformed bacteria cells will grow in the presence of the antibiotic while bacteria cells that did not take up the plasmid, will not grow.



pUC18 Plasmid

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ACTIVITY 6A

Bacterial Transformation - Ampicillin Resistance

Objectives

- ***Understand*** **the techniques used in genetic engineering.**
- ***Demonstrate*** **the process of bacterial transformation.**
- ***Identify*** **transformed bacterial cells.**
- ***Calculate*** **transformation efficiency.**

What You Need

Per Student

Gloves
Apron
Goggles

Per Group

	Marker
2	2-mL Sterile microfuge tubes
	Microfuge tube rack
2	Sterile Micropipets
500 µl	0.05 M $CaCl_2$
1	Sterile inoculating loop
10µl	pUC18 solution
2	Luria broth agar plates
2	Luria broth agar with ampicillin plates
500 µl	Luria broth
2	Spreading rods
	Clear tape

Shared Materials

E. coli culture plate
Ice bath
42°C Water bath
Incubator set at 37°C

Safety

Always follow proper lab safety and aseptic technique protocols. Wear protective gloves, goggles, and a lab apron when working with bacteria. Once your petri plates have been inoculated with bacteria, seal them with clear tape and do not open them.

When working with bacteria, do not touch your face or mouth with your hands. Keep your work area clean. At the end of the investigation, collect all petri plates and all contaminant items such pipets, microfuge tubes, etc. Prior to disposal, your teacher may instruct you to sterilize all contaminated materials by immersing them in a 5% bleach solution for about 1 hour. Work area should be disenfected by wiping it with either a bleach solution or 95% ethanol. Dispose of all materials as instructed by your teacher.

Wash your hands immediately after handling bacteria and before leaving the laboratory.

What to do...

STEP 1

Obtain two poured Luria broth agar plates. Label one of the plates "LB+", and the other plate "LB-".

STEP 2

Obtain two poured Luria broth agar with ampicillin plates. Label one of the plates "LB/Amp+", and the other plate "LB/Amp-".

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STEP 3

Obtain two sterile 2-mL microfuge tubes. Label one of the tubes "+" and the other tube "-".

STEP 4

Using one sterile, plastic 1-mL micropipet, add 250 µl of ice cold 0.05 M $CaCl_2$ to each of the sterile microfuge tubes.

STEP 5

Using a sterile, plastic inoculating loop, add 2-3 *E. coli* colonies to each of the two 2-mL microfuge tubes. Try not to gouge the agar that the *E. coli* are growing on as no agar should be introduced to the tubes. Tap the inoculating loop firmly against the side of each tube to ensure that a colony is introduced to each tube. Discard the inoculating loop as directed by your teacher.

STEP 6

Use the micropipet from Step 4 to suspend the cells in the $CaCl_2$ in the tubes. Do this by alternately drawing the solution from the tube into the micropipet and emptying it several times. Discard the micropipet as directed by your teacher.

STEP 7

Using a digital or fixed volume micropipet, introduce the antibiotic-resistance plasmid by adding 10 µl of pUC18 solution into the "+" tube, and mix the contents by tapping on the tube.

STEP 8

Place both microfuge tubes directly in the ice container for a total of 15 minutes.

STEP 9

Heat shock the bacterial cells by placing both tubes in a water bath at 42°C for a total of 90 seconds.

STEP 10

Return both tubes immediately to the ice container, and allow them to remain there for 2 minutes.

STEP 11

Using one sterile, plastic 1-mL micropipet, add 250 µl of Luria broth to each of the microfuge tubes and tap on each tube to mix the contents. Place the tubes in the microfuge tube rack at room temperature. Discard the micropipet as directed by your teacher.

STEP 12

Using a digital or fixed volume micropipet, draw 100 µl of "+" cells and dispense them onto the "LB+" plate. Place 100 µl of "+" cells on the "LB/Amp+" plate also.

STEP 13

Use a sterile spreading rod to spread the "+" cells across each of the two "+" plates. Cover the plates immediately and place them aside to set for a few minutes. Resterilize the spreading rod with alcohol and flame.

STEP 14

Using a clean tip on the digital or fixed volume micropipet, draw 100 µl of "-" cells and dispense them onto the "LB-" plate. Place 100 µl of "-" cells on the "LB/Amp-" plate also.

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STEP 15

Use a new sterile spreading rod and use it to spread the "-" cells across each of the two "-" plates. Cover the plates immediately and place them aside to set for a few minutes. Resterilize the spreading rod as you did previously.

STEP 16

Tape all plates shut using clear tape. Incubate the plates upside down overnight in a 37°C incubator.

Analysis

Observe each of the incubated plates. Do NOT open them; colonies are visible through the cover of the plates. Count the total number of colonies present on each plate and enter those values in Data Table 1. If the colonies are too numerous to count (if the colonies have grown into each other), this is called a "lawn." Record this in Data Table 1.

Data Table 1

Plate	Number of Colonies
LB+	
LB-	
LB/AMP+	
LB/AMP-	

The total number of antibiotic-resistant bacterial colonies visible on each plate can be used to calculate the transformation efficiency, or the number of antibiotic-resistant colonies/μg pUC18. Perform the following calculations to determine the transformation efficiency.

In this procedure, you used a 0.005 μg/L concentration. You used a total volume of 10 μl pUC18. Calculate the total mass of the pUC18 used:

Total mass of pUC18 (mass = volume x concentration): _____

Total volume of cell suspension: _____

Fraction of the total cell suspension spread on plate (μl spread/total volume): _____

Mass of pUC18 in the cell suspension (total mass x fraction spread on plate): _____

The transformation efficiency is the number of colonies/μg plasmid (# colonies observed/mass of pUC18 in suspension):
_____ (use scientific notation)

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Questions

1. Three of the plates were controls. Which three plates were the controls? For each, describe how it was a control.

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2. Make a pairwise comparison of the following plates: LB+ and LB-; LB+ and LB/Amp+; LB- and LB/Amp-; LB/Amp+ and LB/Amp-. Describe the growth on each pair of plates and discuss why the growth appeared as it did.

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